
Mesenchymal Stem Cell Manufacturing for Clinical Use

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Abstract

Mesenchymal stromal/stem cells (MSCs) are adult, self-renewable, multipotent cells that can be isolated in various tissues, most commonly bone marrow (BM) and adipose tissue. Because of their capacity for self-renewal and differentiation into tissues of mesodermal origin and due to their immunomodulatory ability, MSCs are used in many preclinical and clinical studies as possible new therapeutic agents for the treatment of a very broad range of conditions, including heart, hepatic, and neurodegenerative diseases.

Whatever the tissue of origin or the clinical application, the MSCs must be expanded *in vitro* to obtain sufficient cell numbers, also when multiple doses are needed.

The MSC manufacturing process for clinical use should comply with the principles of Good Manufacturing Practice (GMP). This ensures that cell preparations are produced and controlled, from the collection and manipulation of raw materials, through the processing of intermediate products, to the quality controls, storage, labelling and packaging, and release.

The application of GMP to manufacture medicinal products such as MSCs must ensure that clinical trials are unaffected by inadequate safety, quality, or efficacy arising from unsatisfactory manipulation of the cells.

Keywords: Mesenchymal stem cells, good manufacturing practice, stem cell therapy

1. Introduction

Cell-based therapy has led to the development of new biological medicines to repair, replace, or recover the biological function of damaged tissues and organs [1]. Among cell types used for this purpose, human MSCs are considered as advanced therapy medical products (ATMPs) and should be handled with appropriate controls to ensure their safety, quality, and efficacy

as a final drug [2-3]. Mesenchymal stromal or stem cells have become of great interest in these years in the field of regenerative medicine. They were initially referred to as multilineage progenitor cells isolated and culture-expanded from adult human BM that, once in culture, display a heterogeneous morphology and are capable of several subpopulations. They can be expanded *ex vivo* and readily differentiate into mesodermal cell derivatives [4-5]. Horwitz and the International Society for Cellular Therapy (ISCT) better defined them as “mesenchymal stromal cells” (MSCs) [6].

The translation of research-based protocols into large-scale production of clinical-grade MSCs requires careful analysis to identify and control all critical aspects: source of MSCs and raw materials, culture media, supplements and disposable devices, and quality control tests [7]. The quality and safety of the cell preparations should be ensured by the implementation of a quality system that guarantees the certification and traceability of every batch of material and supply utilized for the procedures, correct utilization and cleaning of instruments, and the locations necessary for stem cell manipulation. Facilities, isolation methods, seeding density, growth factors, and chemicals can all influence the expansion potential and functional properties of MSCs and should be considered throughout the production process. The organization structure, qualification of staff with high levels of expertise, and the appropriate equipment must be implemented in dedicated cleanrooms, currently named “Cell Factories,” in compliance with current good manufacturing practice (GMP) standards. The application of GMPs for aseptic production ensures the safety of the final cell therapy product. In 2008, the International Society for Stem Cell Research released a set of recommended guidelines for the development of stem cell-based treatments [8]. These recommendations are focused on the risks involved with stem cell-based therapies, the voluntary informed consent, the patient monitoring and adverse event reporting, and the equality of benefits of stem cell treatments.

2. Body

MSCs are adult, self-renewable, multipotent cells that can be isolated in various tissues, most commonly BM and adipose tissue, but also in fetal tissues. Because of their capacity for self-renewal and differentiation into tissues of mesodermal origin and due to their immunomodulatory ability, MSCs are used in many preclinical and clinical studies as possible new therapeutic agents for the autoimmune or degenerative diseases treatment [9].

The MSC manufacturing process for clinical use should comply with the principles of GMP. This ensures that cell preparations are produced and controlled, from the collection and manipulation of raw materials, through the processing of intermediate products, to the quality controls, storage, labelling and packaging, and release, according to the expected requirements. During the whole production process, critical steps should be known and described. A thorough risk analysis during all phases of production and control ensures a final product with the expected quality, both for patients and for the regulatory authorities responsible for the controls.

2.1. MSC source

BM is still the most common source of MSCs. However, during the last two decades alternative and more accessible tissue sources of MSCs have been identified.

MSCs have been isolated from fat, deciduous teeth, placenta, and umbilical cord blood, showing comparable features to BM-derived cells [10]. In particular, fresh umbilical cord blood is the third common source for isolating MSCs for clinical use, but the success rate in isolating and further expanding MSCs depends on the volume of blood collected, the cell content, and the time between collection and processing [12]. Related cell sources, including neonatal tissues as amniotic fluid [11], placenta, and Wharton's jelly (WJ), are interesting for their ability to maintain their self-renewal capacity *in vitro* for a long period, showing a higher pluripotency capacity as compared to BM-MSCs [13-15].

Adipose-derived stem cells (ASCs), if compared to BM MSCs, appear to have higher frequencies [16], higher proliferative capacity [16], and to be more stable in culture with a lower senescence ratio [18]. The methods to isolate MSCs from lipoaspirates are standardized, even with automated device to separate the tissue fraction of fat from the contaminating fluids [19]. ASCs have been tested in preclinical studies for the treatment of a variety of clinical conditions, including Crohn's disease, muscular dystrophy, myocardial infarction, spinal cord injury, diabetes mellitus type 1, breast reconstruction, and facial lipoatrophy [20].

2.2. MSC isolation and expansion

Several different methods for isolating MSCs have been described. After harvesting and, if necessary as in case of fat, dissociation of tissue, MSCs could be seeded in flask with or without gradient separation (Ficoll or Percoll), or after immunomagnetic enrichment [21]. The paper of Mareschi showed that direct selection of MSCs from BM cells by adhesion to culture plastic is a more advantageous method compared to MSCs obtained by gradient separation [22], resulting also in a significantly higher level of HLA-DR [23]. Cell-plating density is a crucial issue for MSC expansion. The use of very low density ensures a high proliferative potential [24]. However, plating densities of 10 and 100 cells/cm² did not produce any expansion [22]. Limiting the number of cell population doubling (CPD) to less than 20 avoids the progressive senescence of expanded MSCs [23].

The use of reagents and supplements clinical grade and suitable for clinical application must be considered for the *in vitro* expansion. Only few clinical grade reagents are commercially available. Animal serum-free media, human serum, and other supplements of human origin have been investigated. Human platelet lysate (HPL) has been demonstrated to be safe and efficient as MSC culture supplement for robust MSC expansion and is now considered a good fetal bovine serum (FBS) substitute [25-26]. In particular, Pathogen Inactivated HPL represents a good GMP-compliant alternative to FBS [27].

2.3. MSCs as ATP

The European Regulation 1394/2007 introduced a new classification for cell therapy products as medicinal products: the tissue-engineered products [28]. The Regulation defines Advanced

Therapy Medicinal Products (ATMPs) as the gene therapy medicinal products, the somatic cell therapy medicinal products, and the tissue-engineered products. These products have specific pharmacologic, metabolic, and immunologic activities and the potential for treating a variety of disorders. For these reasons, cellular products for ATMPs, prepared on a routine basis, must meet the same stringent conditions required for drugs preparation and commercialization. In particular, they must be manipulated according to the GMP [29], and they require preclinical testing in Good Laboratory Practice (GLP) [30-31] and clinical trials conducted in Good Clinical Practice (GCP) before being commercialized [32].

MSCs represent a promising strategy for the development of new therapeutic strategies due to the numerous applications proposed for their use [33]. Thanks to their intrinsic properties, MSCs represent an attractive candidate for clinical applications [34-36]. As ATMPs, MSCs also must satisfy all the above-mentioned requirements. The expansion protocol of MSCs for clinical use should comply with the principles of GMP and take in account all critical steps of the process. The quality and safety of the expanded MSCs must be maintained throughout their production and quality control cycle, ensuring their safe use in the patient. The main critical steps are the collection and manipulation of raw materials; the processing of intermediate products; the quality control tests; storage, labelling, and packaging; and release. The validation of the process and a thorough risk analysis during all phases of production ensures a final product with the expected quality, both for patients and for the regulatory authorities responsible for controls [37].

2.4. GMP production of MSCs

MSCs represent a small fraction (0.001-0.01%) of the BM cell population; therefore, to obtain a sufficient number of cells, they must be extensively *ex vivo* expanded.

One of the most significant properties that make MSCs a special device for cell-based therapeutic approaches is their capability to expand in culture for several passages, without compromising their pluripotent potential. MSCs can easily be expanded in culture thanks to their capacity to adhere and proliferate maintaining their immunophenotypic characteristics and functions as multipotent cells.

Several papers discussed in the last years the standardization of MSC culture conditions for clinical use. As reported in the review by Ikebe, a total of 47 reports, describing MSC-isolation methods, were found in literature published from January 2007 to 2013 [38].

The recent paper of Wuchter summarizes a consensus meeting between researchers, clinicians, and regulatory experts on standard quality requirements for MSC production [39]. The manufacture of sterile products, such as the ATMPs, is subject to special requirements in order to minimize the risks of microbiological contamination. Maintaining the cells in culture for several passages means subjecting them to the risk of contamination. The production process of MSCs must be performed through a GMP protocol based on standard operating procedures (SOPs) and documented according to GMP [40-41].

As mentioned in Annex 1 of GMP [42], the manufacture of sterile products should be carried out in clean areas, laboratory named cleanrooms or cell factories, in which the concentration

of airborne particulates is maintained and controlled. The key elements for designing a cleanroom according to GMP requirements are the presence of HEPA filters, a room air change rate of >20 per hour, the temperature and humidity control, and the air pressure differential. The appropriate cleanliness standard can be maintained also through an appropriate management of personnel, equipment, and materials. Premises and equipment should be designed in order to minimize the accumulation of particles or microorganisms and to permit the appropriate cleaning.

For the manufacture of sterile medicinal products, four grades can be distinguished:

- Grade C and D: clean areas for carrying out manufacturing steps not so critical.
- Grade B, for aseptic manipulation, is the background environment for grade A.
- Grade A, the local zone for high-risk operations, where the product is manipulated in an open system. This condition is normally the laminar airflow biosafety cabinet.

Annex 1 also gives the recommended limits for microbiological monitoring of clean areas during aseptic operations.

The concept of quality assurance and quality management are related to GMP requirements.

As reported in the Chapter 1 of GMP guidelines [43], the quality management covers all matters, which individually or collectively influence the quality of a product.

A GMP-compliant quality system for the manufacture of medicinal products should ensure that cell preparations are controlled, from the collection and manipulation of raw materials, through the processing of intermediate products, to the quality controls, storage, labelling and packaging, and release. During the whole production process, critical steps should be known and described.

An appropriate quality system should cover all the following aspects:

- Roles and responsibilities. All the staff must be qualified and adequate. A qualified person must certify the release of the medicinal products. Competencies, training and retraining must be scheduled.
- Premises and equipments.
- Documentation.
- Raw materials, reagents, and suppliers.
- Quality control on intermediate products, in-process controls, and validations.
- Activities in outsourcing.
- Deviations, corrective/preventive actions, and change control.
- Storage, distribution, and labelling.
- Self-inspection and/or quality audits.

Good documentation constitutes an essential part of the quality assurance system. Documents may exist as paper-based or electronic format. The main documents, as required by GMP are as follows:

- Site Master File, describing the Facility and the GMP activities.
- SOPs, detailed description of various processes.
- Working instructions, giving instructions on technical methods or activities.
- Records, registration, and reports, providing evidence of actions and steps.
- Certificate of analysis, providing a summary of quality control tests with the evaluation of compliance of the product batch.

Approved procedures and instructions must be written in a clear and unambiguous form, and the records must demonstrate that all the steps required by the defined procedures and instructions were carried out by qualified operators.

2.5. Quality control

Quality Control is that part of GMP which is concerned with sampling, specifications, and testing [44], also including outsourcing activities. The quality control process should be validated to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

The recent paper of Torre and colleagues provides the minimal quality requirements for the MSC production and its delivery for clinical use, to guarantee the safety of the final cell therapy product. For this purpose, the document evaluates the most important steps of GMP-compliant MSC production: the isolation and expansion process; the validation phase of the process, including all quality controls for the characterization, functionality, potency, and safety of MSCs; and the quality control at the batch release to guarantee the safety of patient infusion [37].

An interesting approach to the GMP is validation. The production process should follow a validation protocol, also considering instruments, supplies and reagents, and defining roles and responsibilities of each step [45]. A validation protocol on a minimum of three expansion procedures should be implemented to standardize the best culture condition and to demonstrate the safety and feasibility of the production protocol. It is a requirement of GMP that manufacturers identify what validation work is needed to prove control of the critical aspects of their particular operations. Significant changes to the facilities, the equipment, and the processes, which may affect the quality of the product, should be validated. A risk assessment approach should be used to determine the scope and extent of validation.

According to International Conference on Harmonization Q2 (ICH Q2) Guidelines [45] and the European (EU) Pharmacopoeia [47], the quality control (QC) process should be validated to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

Quality control analytical methods could be validated demonstrating the accuracy, the specificity, the limit of detection, and the precision [45, 48-49].

In the validation phase of the process, additional assays to test immunomodulation, senescence, and genomic stability, clonogenicity should be carried out. Although tumour formation during the validation process has not been reported in ongoing clinical trials using MSCs [50], tumorigenesis and karyotype [23, 51] should be also tested during validation.

2.6. Clinical trials

Today, numerous MSC preparations from academic institutions and private companies are being investigated. The review paper of Wang summarized the currently completed clinical trials, registered with clinicaltrials.gov, using MSC to treat various diseases. The first clinical trial using culture-expanded MSCs was carried out in 1995 and 15 patients became the recipients of the autologous cells [52]. Since then, a great number of clinical trials have been conducted to test the feasibility and efficacy of MSC therapy.

Today more than 450 clinical trials testing MSC are currently published on the international registry www.clinicaltrials.gov, both recruiting or completed. Most trials are Phase I (safety studies) or Phase I/II studies. Only a small number of these trials are in Phase III.

Expanded MSCs have been used to treat several diseases, from bone/cartilage disorder, cardiovascular diseases, autoimmune diseases, and neurodegenerative disorders [12].

The experience of our group has been focalized to research a new possible strategy for amyotrophic lateral sclerosis (ALS) aimed at cell replacement and neuroprotection.

The long term study of showed the safety of MSC transplantation in the central nervous system during a follow-up of nearly 9 years, and is in support of applying MSC-based cellular clinical trials to neurodegenerative disorders [53-55].

The application of GMP to the manufacturing of investigational medicinal products for clinical trials must ensure that trial subjects are not placed at risk, and that the results of clinical trials are unaffected by inadequate safety, quality, or efficacy arising from unsatisfactory manipulation of the cells. Annex 13 of GMP guidelines is the reference guide for this step [56].

3. Conclusion

Stem cell therapy has evolved over the past years, thanks to the great efforts of researchers to define and implement new methods for MSC expansion, according to ATMP requirements.

The standardization of protocols and procedures, both for production and quality control, within the collaboration of different groups, will lead to the definition of standardized protocols to be considered during the process of expansion and release of MSC as ATMPs for the application of clinical trials with MSCs.

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